EMBO reports

meeting report

Viruses in control of the immune system

Workshop on molecular mechanisms of immune modulation: lessons from viruses

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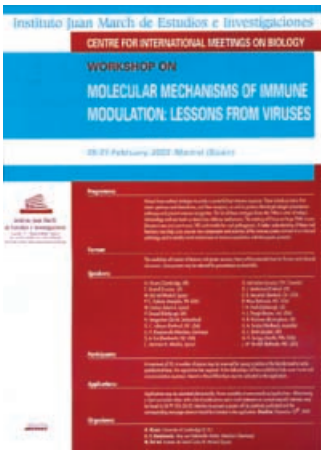
Received June 26, 2002; revised September 4, 2002; accepted September 6, 2002



This Instituto Juan March Workshop was organized by Antonio Alcami, Marga del Val and Ulrich Koszinowski and was held under the auspices of the Instituto Juan March de Estudios e Investigaciones in Madrid, Spain, February 25–27, 2002.

Introduction

Viruses have evolved strategies to evade the inflammatory and immune responses that their hosts have co-evolved to limit viral impact on reproductive fitness (Alcami and Koszinowski, 2000; Tortorella *et al.*, 2000). The number of viral immune evasion strategies identified has increased dramatically in recent years. These rely on proteins that mimic or target specific components of the immune system or that prevent immune recognition of virally infected cells. We are only just beginning to comprehend the subtleties of virus–host interactions, which is of obvious importance for the understanding of viral pathogenesis.

This workshop brought together scientists who view the interactions between viruses and host defence mechanisms from different perspectives. The aim was to discuss strategies of immune modulation employed by viruses, focusing primarily on two large DNA virus families—herpesviridae and poxviridae—whose members are particularly adept at manipulating immunity, and human immunodeficiency virus (HIV), which is highly successful in modifying the immune system despite its smaller genome (Table I). Particular emphasis was placed on gleaning insights into the normal functions of the immune system based on the viral immunomodulatory effects. We are rapidly approaching a time when we will be able to apply this knowledge to design better vaccines and treatments for viral infections and autoimmune diseases.

Viruses and hosts: an intimate relationship

Infectious agents exist in a dynamic equilibrium with their hosts in which both immune and non-immune pathways contribute to stringent control, resulting in viral clearance and/or asymptomatic homeostasis (Ghazal *et al.*, 2000). In the light of this, an infection can be viewed as a highly complex and intricate dance that takes place between two partners—the host and the pathogen. As part of this dance, the immune system is often not completely efficient in clearing the virus, and viruses have evolved both general and specific strategies to counteract immune attack. During a primary infection, after several weeks of innate and acquired B- and T-cell-mediated clearance, the residual population of virus may eventually be eliminated or adopt one of two viral life-cycle strategies. These two strategies are represented by states of either viral persistence (continuous replication) and/or latency (a reversible non-productive infection) in the host. In both cases, viral replication is kept in check by a secondary tier of control exerted at the level of the infected cell, which is superimposed on the infection as the virus reaches a state of homeostasis with the host (Figure 1). Regulation at this level is modulated by signalling pathways, apoptosis and the cell cycle.

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Table I. Some of the viruses and immune evasion strategies discussed in this report

Virus	Family (genome)	Protein	Function/mechanism
Vaccinia virus (VV)	Poxvirus (DNA)	Interleukin 18 (IL-18) binding protein (IL-18BP)	Secreted protein that binds IL-18 and neutralizes its activity
Molluscum contagiosum virus (MCV)	Poxvirus (DNA)	IL-18BP	Secreted protein that binds IL-18 and neutralizes its activity
Ectromelia virus (EV)	Poxvirus (DNA)	CD30	Secreted protein that binds CD30 ligand
Fowlpox virus (FPV)	Poxvirus (DNA)	Interferon-γ (IFN-γ) receptor	Secreted protein that binds IFN-γ, unrelated to host receptor
Yaba-like disease virus (YLDV)	Poxvirus (DNA)	Viral chemokine receptors (vCKRs)	Membrane protein that binds chemokines
Human cytomegalovirus (HCMV)	Herpesvirus (DNA)	US28	vCKR
		US27, UL33	vCKR homologues
		US2, US11	Degrade MHC class I molecules
		US3, US10	Interference with MHC class II presentation
			Upregulation of CD95L and TRAIL and induction of apoptosis in T cells
Murine cytomegalovirus (MCMV)	Herpesvirus (DNA)	Viral chemokine (vCK)	Chemoattractant properties
		M04, M06	Downregulation of MHC class I
		M152	Interferes with MHC class I presentation and NK cell activation
Kaposi's sarcoma-associated herpesvirus (KSHV)	Herpesvirus (DNA)	vCKR ORF74	Membrane protein that binds chemokines, consitutively activated, implicated in the development of Kaposi's sarcoma lesions
Murine γ-herpesvirus 68 (MHV-68)	Herpesvirus (DNA)	Viral chemokine binding protein (vCKBP) M3	Secreted, binds and neutralizes chemokines
α-herpesviruses	Herpesvirus (DNA)	vCKBP	Secreted, binds and neutralizes chemokines

Vital issues surrounding the molecular and cellular intimacy of this dance were underscored by an eclectic group of presentations. H. Hengartner (Zurich, Switzerland) contrasted infections caused by the non-cytopathic lymphocytic choriomeningitis virus (LCMV) and the cytopathic vesicular stomatitis virus (VSV) in terms of the type of antiviral response elicited. He presented evidence that different arms of the immune system are triggered:

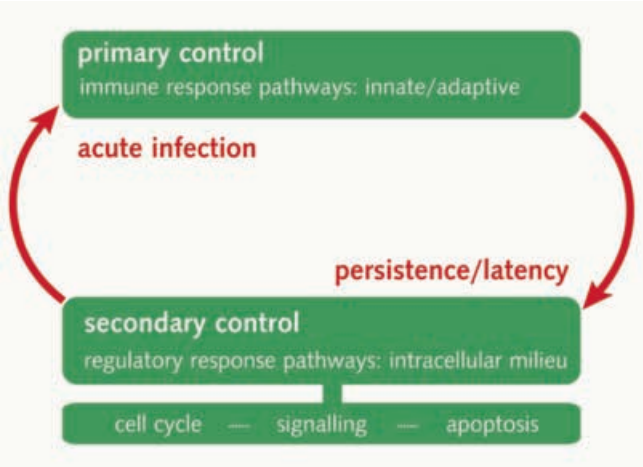

Fig. 1. Homeostatic control of infection. Primary control of viral infection is exerted by immune responses. Dependency on host factors provides a secondary level of control.

cytotoxic T lymphocytes (CTLs) in the case of LCMV, and neutralizing antibodies for VSV. Neutralizing antibodies were also shown to have an important role in the long-term immune control of persistent LCMV infection. He also presented evidence strongly implicating antigen persistence in the maintainance of B-cell memory. In a related talk, G. Karupiah (Canberra, Australia) addressed the role of cytokines, CTLs and antibodies in protecting against cytolytic poxvirus infections. In the case of the highly virulent ectromelia virus (EV), both CTLs and antibodies were clearly required. The role of antibodies was unexpected, because they have traditionally been implicated in the prevention of re-infection rather than in the initial clearance of virus. Interferon (IFN)-γ, a cytokine that promotes cell-mediated immunity, appeared to play a more important role in the case of the attenuated vaccinia virus (VV) than against the virulent EV. Overall, these talks argued against the idea that viruses engage 'general' immune responses, suggesting instead that they have each evolved to elicit a designed sculpting of the immune response that is tailored to the specific pathogen.

P. Ghazal (Edinburgh, UK) addressed the issue of whether the strict dependence of a virus on host-cell signalling pathways and synthetic machinery could be exploited by immune and cellular responses to infections. He presented data from transgenic mice expressing β-galactosidase in their retinal cells under the control of the human cytomegalovirus (HCMV) major immediate early promoter, which showed that only those cells in which the promoter was active supported HCMV replication. This illustrates that a non-immune-mediated signalling pathway controls

virus replication in the retina and thus the development of congenital HCMV retinitis. The idea that cellular control pathways may restrict a productive infection was extended to immune-mediated responses using comparative microarray studies, which measured global changes in cellular gene expression upon HCMV infection and in response to IFN. A mathematical theoretical model for understanding quantitative gene interactions in limiting the viral infection was presented.

The importance of host genes and genetic background for viral propagation was further highlighted by T. Scalzo (Nedlands, Australia), who described elegant mouse genetic studies addressing the role of the natural killer gene complex (NKC) in controlling murine cytomegalovirus (MCMV), herpes simplex virus (HSV) and EV infections. The NKC encodes many cell surface molecules that are predominantly expressed on NK cells and function as either activation or inhibitory receptors. In the case of MCMV, Ly49H is an activation receptor that contributes to a 3–4 log difference in viral titres in the spleen and bone marrow. Other genes within the NKC are specifically involved in controlling innate responses to HSV and EV infections, revealing an array of mechanisms that genetically determine immune control of distinct viruses in this locus.

The power of genetics was also demonstrated by U. Koszinowski (Munich, Germany), who used a viral genetics system to explore the intricate relationship between host and virus. His group engineered an array of combinatorial mutants for the three major genes (M04, M06 and M152) of MCMV that are involved in downregulating the major histocompatibility complex (MHC) class I presentation, and they uncovered a well-defined hierarchy of cooperation and antagonism between them. These results NSprovide a functional rationale for the evolution of multiple immunomodulatory genes for fine-tuning the host response.

Viral modulation of cytokine and chemokine networks

Cytokines are small proteins that provide a complex system of cellular intercommunication. They play a key role in the initiation and regulation of the immune response, and some of them, such as IFNs and tumour necrosis factor (TNF), induce intracellular pathways that activate an antiviral state or apoptosis and limit viral replication. It is therefore not surprising that viruses have learned how to inhibit the production and activity of cytokines (McFadden and Murphy, 2000). Chemokines are chemoattractant cytokines that control leukocyte migration through the body and their infiltration into tissues during inflammation. More than 40 chemokines have been identified and grouped into four classes according to the number and spacing of their cysteine residues: CC, CXC, C and CX3C. Chemokine receptors, of which 16 have been identified, are expressed in different cell subsets and determine which leukocytes predominate during a particular immune response. One of the viral anti-cytokine mechanisms identified in recent years is the production of viral molecules that mimic cytokines, chemokines or their receptors (Figure 2). The function of many of these viral molecules in the context of infection is largely unknown. It is thought that they contribute to the evasion of specific immune responses or promote viral replication, but they may also have pathological effects in the host.

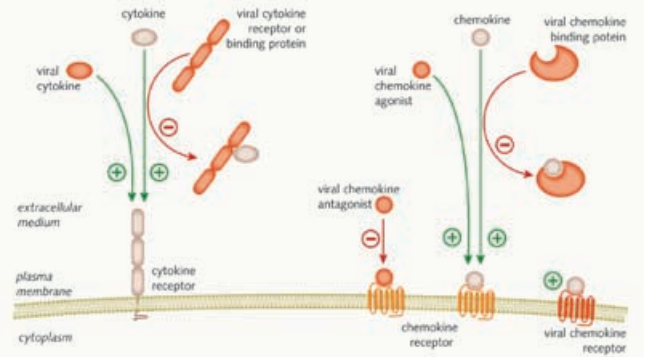

Fig. 2. Cytokines and cytokine receptors encoded by viruses. Viruses encode cytokine and chemokine homologues that bind to specific receptors and either trigger signal transduction and biological responses (agonist) or occupy receptor binding sites and prevent binding of host cytokines (antagonist). Viruses also encode homologues of seven-transmembrane-domain chemokine receptors that are expressed at the surface of infected cells and may secrete proteins that bind with high affinity to host cytokines or chemokines and neutralize their activity. These may have sequence similarity to the extracellular domains of cellular cytokine receptors (viral cytokine receptors) or have unique sequences (viral cytokine/chemokine binding proteins).

IL-18 is a pro-inflammatory cytokine required for IFN-γ production. Its biological activity is controlled by a secreted binding protein, IL-18BP, which has no sequence similarity to known membrane receptors. Several poxviruses encode IL-18BP homologues. B. Moss (Bethesda, MD) showed that the IL-18BP encoded by molluscum contagiosum virus (MCV) has a lower affinity for IL-18 than the human IL-18BP. This was unexpected, since other viral cytokine receptors have binding affinities similar to, or even higher than, those of their cellular counterparts. It was speculated that this may prevent MCV from causing immunosuppression in the host while inhibiting IL-18-induced immunity to some extent. An extended C-terminal domain in the MCV IL-18BP, which is absent in other IL-18BPs, has no described function to date. Moss, however, showed that this domain binds to heparin and it may therefore anchor the IL-18BP at the cell surface and maximize its inhibitory activity in the vicinity of the infected cell. This illustrates how viruses can copy a cellular protein and modify its properties for their own benefit. G. Smith (London, UK) showed that inactivation of the VV IL-18BP increased the production of IFN-γ and the activities of NK cells and CTLs in infected mice. Thus, the VV IL-18BP protects the virus from the cellular immune responses known to specifically target it.

Four genes encoding soluble TNF receptor homologues have been identified in poxviruses. A. Alcami (Cambridge, UK) described the identification of another TNF receptor superfamily member, a homologue of CD30, encoded by EV. The viral CD30 is secreted from infected cells, binds the CD30 ligand and blocks inflammatory responses mediated by type 1 cytokines, such as IFN-γ and IL-12, which promote potent antiviral responses mediated by CTLs and NK cells. This is the first poxvirus cytokine receptor found both to act as a decoy receptor and to induce reverse signalling in cells expressing the ligand. These studies illustrate that the characterization of viral proteins may uncover novel functions of their human counterparts.

In some instances, viruses 'invent' their own molecules instead of copying them from their hosts. This was illustrated by F. Puehler (Freiburg, Germany), who described a soluble IFN-γ receptor of novel structure encoded by fowlpox virus (FPV). This contrasts with the other poxviruses, which encode a homologue of the human IFN-γ receptor that may have been acquired from the host.

Viruses may modulate the chemokine network by encoding homologues of chemokines (vCKs) or chemokine receptors (vCKRs) or by secreting chemokine binding proteins (vCKBPs) that have no sequence similarity to host proteins. The role of vCKs in attracting leukocytes to the site of infection to enhance virus dissemination was highlighted by E. Mokarski (Stanford, CA). He compared wild-type MCMV with mutants lacking the *mck* gene, which encodes two forms of vCKs, and found that the vCKs increased inflammatory responses, but that this increased innate response did not affect the rate of virus clearance at the site of inoculation. However, virus dissemination (viremia and salivary gland replication) was increased.

The function of vCKRs during viral infection is difficult to predict. The identification of two vCKRs in the genome of the poxvirus Yaba-like disease virus (YLDV) was discussed in another part of Smith's presentation. These viral proteins are closely related to CCR8, a chemokine receptor that binds the CC chemokine I309 and is expressed in monocytes and Th2 cells. It was found that one of the YLDV vCKRs binds I309 and other chemokines from the CC class. Understanding why viruses have 'stolen' one particular chemokine receptor from among the many encoded by their hosts will be very informative. The intracellular localization of the chemokine receptor-like proteins encoded by HCMV (US28, US27 and UL33) was discussed by A. Fraile-Ramos (London, UK). When visualized by immunogold electron microscopy, these receptors were mostly localized to multivesicular endosomes, with a fraction expressed at the cell surface. This localization to endosomes may allow these proteins to be incorporated into the viral envelope during HCMV assembly.

The role of vCKRs in promoting pathology was elegantly illustrated by S. Lira (Kenilworth, NJ). Transgenic mice expressing the vCKR from Kaposi's sarcoma-associated herpesvirus (KSHV), ORF74, in the absence of viral infection, causes the development of Kaposi's sarcoma-like lesions characterized by increased vascularization and recruitment of inflammatory cells. The fact that very few cells in the lesions of these mice express ORF74 is reminiscent of Kaposi's sarcoma lesions where only a few cells are infected with KSHV and suggests a paracrine effect. The signal transduction capacity of ORF74 is modulated by chemokines and triggers a series of events leading to the development of pathology.

The vCKBPs were initially described in poxviruses, and the murine γ-herpesvirus 68 (MHV-68) M3 protein was the first herpesvirus-encoded vCKBP to be identified. Lira showed that, in transgenic mice expressing the chemokine secondary lymphoid chemokine (SLC) and/or M3 in the pancreas, M3 efficiently blocks the activity of the former. SLC controls the migration of lymphocytes into lymphoid organs and lymphoid neogenesis, and inhibition of this activity may be important for MHV-68 in evading the immune response. Whereas vCKs and vCKRs have been described in β- and γ-herpesviruses, limited information is available on the modulation of chemokine activity by α-herpesviruses, which may use different tricks. However, Alcami described the identification of a novel family of vCKBPs encoded by α-herpesviruses that bind a broad range of chemokines and neutralize their ability to induce cell migration and, presumably, the infiltration of immune cells into infected tissues. This is the second family of vCKBPs to be identified in herpesviruses, and they have no sequence similarity to other vCKBPs or host chemokine receptors.

Viral interference with cellular immunity

Elements of the cellular immune system often play crucial roles in host responses to viruses (Yewdell and Bennink, 1999; Xu *et al.*, 2001). These elements can be split into two categories: innate immune cells (macrophages, granulocytes and NK cells) and adaptive immune cells ('helper' T_{CD4+} cells and the cytotoxic T_{CD8+} cells). Innate immune cells are triggered by general alterations associated with viral infections, whereas T cells are triggered by viral peptides recognized in association with MHC class II (T_{CD4+}) or class I (T_{CD8+}) molecules. Naïve T cells (i.e. before their first exposure to antigen) are activated by professional antigen presenting cells (APCs) such as dendritic cells (DCs), which possess co-stimulatory molecules necessary for such activation. Only the adaptive immune response demonstrates memory, and this property is exploited by vaccines that elicit T-cell immunity in order to provide protection against subsequent infection.

A crucial question for viral immunology and vaccine development is how viral or vaccine antigens are presented to T cells *in vivo* and how viruses might interfere with this process (Figure 3). J. Yewdell (Bethesda, MD) showed that, while VV infects both DCs and macrophages in lymph nodes draining the site of infection, virus-specific T_{CD8+} cells interact only with the infected DCs. T_{CD8+} cells also interact with uninfected DCs, which may have been presenting viral antigens acquired from infected cells, a phenomenon known as cross-priming. To determine the extent of cross-priming, Yewdell's group engineered VV to express the HCMV US2 or US11 glycoproteins, which degrade MHC class I molecules, so that the infected cells would not be presenting endogenous viral antigens on their surface. They found this resulted in only a partial decrease in the T_{CD8+} cell response, with most of the residual responding T_{CD8+} cells apparently being induced by a subset of viral antigens presented on non-infected DCs via the cross-priming route, which would not be affected due to the *cis*-acting nature of the VV-expressed HCMV glycoproteins.

The importance of cross-priming in eliciting T_{CD8+} cell responses was underscored by A. Hill (Portland, OR). Like HCMV, MCMV encodes several proteins that interfere with MHC class I molecule function, possibly to cope with the hundreds of class I genes present in mouse populations. The function of some of these gene products can be completely inhibited by a single viral protein, while others require the concerted action of multiple immune evasion molecules. These requirements vary in a cell-type-dependent manner. One such interfering protein, m152, completely inhibited the presentation of a defined MCMV peptide *in vitro*, but it had no effect on the induction of T_{CD8+} cells specific for this determinant *in vivo*. This may be due to its efficient presentation by the cross-priming pathway. Alternatively, m152 may not be effective in APCs that

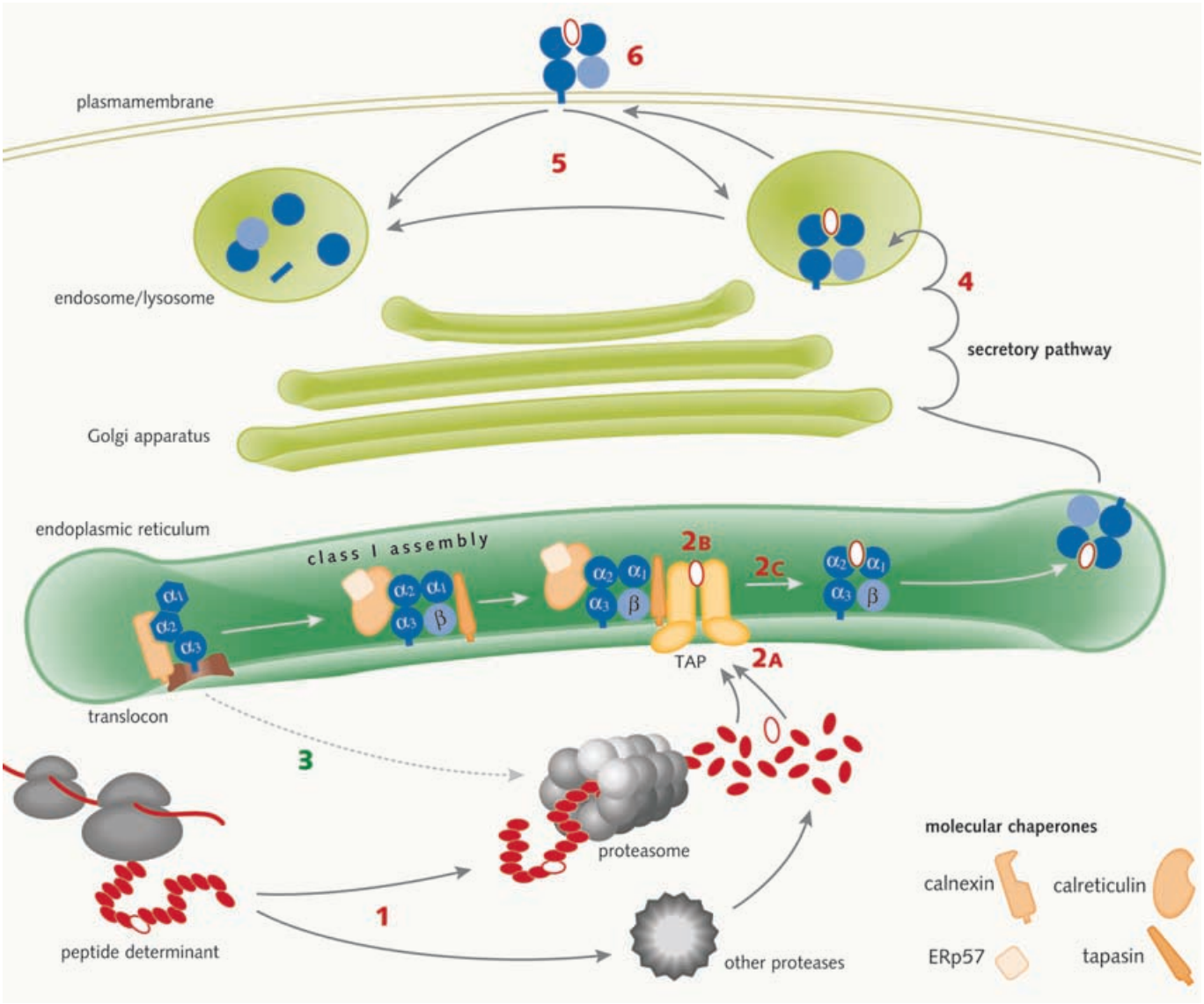

Fig. 3. The classical class I pathway is depicted with reference to viral interfering proteins. Oligopeptides (white ovals) are derived from defective ribosomal products and other cytosolic substrates through the action of proteasomes and other endopeptidases acting in concert with aminopeptidases that trim oligopeptides in the cytosol and the endoplasmic reticulum (ER). Class I heavy chains (α_1, α_2 and α_3) and β_2m (shown as β) are translocated into the ER, where they fold with the help of the molecular chaperones indicated. Heterodimers bind to transporters associated with antigen processing (TAP) via the dedicated chaperone tapasin until oligopeptide binds, which releases class I molecules, enabling them to make their way to the cell surface via the standard default vesicular trafficking pathway. Viral proteins interfere with this process at multiple steps. (1) Epstein–Barr virus nuclear antigen (EBNA)-1 contains a sequence that renders it resistant to proteasomal degradation. HCMV IE is phosphorylated in infected cells by a virion capsid protein, somehow preventing antigen processing. (2A) HSV ICP47 and a bovine herpesvirus protein bind to the cytosolic side of TAP and prevent peptide translocation. (2B) HCMV US6 binds to TAP in the ER lumen and prevents peptide translocation. (2C) Several viral proteins bind to class I molecules in the ER, retaining them and/or interfering with the function of the peptide loading complex. The proteins include adenovirus E319K (also prevents tapasin-mediated docking of class I with TAP), HCMV US3 and MCMV m4/gp34. (3) HCMV US2 and US11 and MHV-68 K3 bind class I molecules in the ER, inducing retrograde transit through translocon for degradation by cytosolic proteasomes. HIV-1 Vpu also induces degradation of newly synthesized class I, probably via translocation to the cytosol. (4) MCMV m152/gp40 causes class I molecules to be retained in the ER *cis* Golgi complex intermediate compartment. (5) Several viral proteins remove class I molecules either from the Golgi or the cell surface. These include MCMV m6/gp48, HIV and simian immunodeficiency virus (SIV) nef, and KSHV K3 and K5. (6) MCMV m4/gp34 is found associated with class I at the cell surface and seems to inhibit T_{CD8+} cell recognition.

present the antigen to T_{CD8+} cells *in vivo*. In either case, this demonstrates the principle that class I-interfering proteins may in some cases act exclusively to inhibit the effector function rather than the induction of T_{CD8+} cells. This may explain how some viruses (e.g. HCMV and HIV) persist in the presence of robust T_{CD8+} cell responses.

D. Johnson (Portland, OR) reported that, as well as removing host MHC class I molecules, HCMV US2 (but not US11) is also capable of destroying MHC class II molecules, although with only approximately half the efficiency. HCMV appears to be rather interested in class II molecules, since it encodes at least two other proteins, US3 and US10, that can interfere with class II-mediated

antigen presentation. US3 appears to act by binding to class II molecules and interfering with its interaction with the invariant chain, which plays an important role in class II assembly and intracellular trafficking. Johnson reported that swapping the transmembrane and cytoplasmic domain of US2 with those of US3 enables the US3 lumenal domain to destroy class II molecules. If these domains of US2 generally enable the destruction of endoplasmic reticulum (ER) ligands that bind to a genetically fused lumenal domain, this could be put to many uses.

H. Ploegh (Boston, MA) presented X-ray crystallographic data revealing precisely how US2 interacts with class I molecules. US2 possesses an immunoglobulin fold with an unusually located disulfide bond, which may enable the structures of related herpesvirus proteins to be modelled. Another interesting feature of US2 is that its N-terminal membrane insertion sequence is neither cleaved nor used as a membrane anchor. US11 is also unusual in that cleavage of its N-terminal signal requires the presence of a C-terminal membrane anchor. Ploegh discussed how US2 and US11 deliver class I molecules to cytosolic proteasomes. Initially, this entails polyubiquitylation of Lys residues in the cytoplasmic domains of class I molecules that possess a folded lumenal domain. It is generally thought that the translocation of ER substrates to the cytosol requires their unfolding before they are able to pass through the translocon. Surprisingly, Ploegh showed that at least a fraction of lumenal GFP genetically fused to class I molecules that are translocated to the cytosol retained fluorescence, indicating either that this folded protein is somehow shuttled into the cytosol (an explanation favoured by Ploegh) or that GFP can refold once it is transported.

Viruses that downregulate cell surface class I molecules must also have strategies for blocking the activation of NK cells, which can detect decreased expression of class I molecules on the cell surface. S. Jonjic (Rijeka, Croatia) reported that MCMV m152, in addition to blocking expression of class I molecules, prevents NK cell activation by reducing the expression of ligands for the NKG2D receptor. Notably, in the past few years, the interaction of viral immunomodulatory proteins with multiple cellular targets has emerged as a common immune evasion strategy.

Concluding remarks

This workshop provided an excellent opportunity to discuss recent progress on the interaction of viruses with the immune system. Consistent with the fine reputation that the Juan March Institute has built over the years, the organization of the meeting was impeccable. Due to the intimate size (50 participants) and high quality of the presentations, there were many illuminating discussions and interactions. The take-home message was clear: understanding virus–host interactions provides a unique opportunity to uncover basic molecular processes and strategies to modulate these pathways. No doubt this will be a fertile area of research for many years to come.

Acknowledgements

We apologize to the many participants whose work has not been discussed due to space limitations. We thank Marga del Val and Ulrich Koszinowski for contributing to the organization of the stimulating scientific sessions. We also thank the Juan March Institute for funding this workshop and providing excellent facilities, and Andres Gonzalez and his team for their outstanding organization.

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DOI: 10.1093/embo-reports/kvf200